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Electrochemical Biosensors for Determination of Ascorbic Acid

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ABSTRACT:
This review introduces how vitamin C (ascorbic acid) is essential for human health and also describes the approaches by which vitamin C can be determined in the different samples. A brief comparison between conventional analytical methods and electrochemical based methods are highlighted in this review. Electrochemical methods based on the immobilized enzymes are preferred over other methods because they are simple, more accurate, highly sensitive and selective as well as inexpensive. Various biosensors are constructed in this field to provide important information regarding ascorbic acid. Early diagnosis of certain disease can be achieved by using ascorbate biosensors. This paper describes the characteristics of most of the electrochemical ascorbic acid biosensors reported till date.

Keywords: Electrochemical biosensor, ascorbic acid, antioxidant, electrode materials, ascorbate oxidase, carbon nanotubes.

INTRODUCTION

Water-soluble vitamin commonly known as vitamin C (L-ascorbic acid), is an essential component of foods because of its nutritional values. Ascorbate (an ion of ascorbic acid) is also act as antioxidant and therapeutic agent. It is one of the most ubiquitous vitamins which were first isolated by Nobel Prize winner, Dr. Albert Szent-Gyorgyi in 1928. With its antioxidant property, it plays a major role as free radicals scavenger and provides defense system to the body against Reactive Oxygen Species (ROS) by preventing the tissue damage [1]. Besides its role as antioxidant, vitamin C is also considered as an effective antiviral agent [2]. It also takes part in the wide range of essential metabolic reactions for better functioning of the body. It is found that vitamin C is important for every body process from bone formation to scar tissue repair [3]. Lack of this vitamin in the body may cause deficiency disease commonly called Scurvy or Scorbutus [4] and in past, many peoples especially sailors were died due to this disease. Most of the species of plants and animals are capable to synthesize the vitamin C. But there are several organisms which do not synthesize vitamin C include- humans, guinea pigs, bats, capybaras, tarsiers and monkeys. Therefore, human beings take ascorbic acid from exogenous sources like fruits and vegetables, can also intake in the form of food supplements as well as pharmaceuticals preparations [5]. Ascorbic acid (vitamin C) is mainly present in berries, citrus fruits, guavas, melons, papayas, green leafy vegetables, tomatoes, cabbage, broccoli, cauliflower, leaf lettuce, and beans [6]. Several investigators reported that some tropical foods are also used as a good source of ascorbic acid. With all its miracle properties, ascorbic acid is widely used in the treatment of disease from scurvy to cancer as well as infertility [7]. The use of ascorbic acid in pharmaceutical products, chemicals, cosmetics, food industry and other natural samples is increases day by day. Therefore, it is necessary to develop a fast, accurate, reliable and easy-to-implement method for determination of ascorbic acid levels in the sample.

Numerous analytical techniques have been reported in the literature for the quantitative analysis of ascorbic acid in fruit juices, urine, plasma, pharmaceutical formulations and in other matrices. These methods includes spectrophotometry [8], titration [9], fluorimetry [10], complexometry [11], turbidimetry [12], flow analysis [13], liquid chromatography [14], HPLC [9] and enzymatic [15]. These methods are less preferred due to certain drawbacks which includes- lack of sensitivity/ and or selectivity, tedious sample preparation steps for removal of interfering compounds [16], overestimation of ascorbic acid level due to the oxidation of several species other than ascorbic acid present in the sample, low minimum detection limit and finally costly. Therefore, electrochemical methods based on enzyme have been preferred over other methods because they are simple, easy-to-use, inexpensive, highly specific and sensitive. Ascorbate oxidase is the enzyme used for these methods. The main problem with these methods lies in the use of free enzyme which increases the cost of procedure. This problem can be overcome by using the method of enzyme immobilization onto the different insoluble supports, which enhances the reusability of enzyme through immobilization.

ELECTROCATALYTIC OXIDATION OF ASCORBIC ACID

Ascorbic acid and the dehydroascorbic acid with two e^-, is the oxidation product of ascorbic acid, in a pH-neutral solution, represent a quasireversible redox reaction coupled with a redox potential of E'_0 = +0.058V versus RHE (Reversible Hydrogen Electrode). Ascorbic acid when present in an aqueous solution shows two deprotonation steps whose p*K*a values are 4.17 and 11.57. Ascorbate anion is the monodeprotonated state of ascorbic acid exists in neutral solution. In a solution with

neutral pH, ascorbate can be oxidized electrochemically by applying potential at inert electrodes (e.g., platinum or glassy carbon). Plenty of oxidizable species are present in real analyte solutions, at a relatively high electrode potential they are oxidized anodically. Substantial bias may arise due to the anodic current, concerned with electro-oxidation of these substances and can exceed anodic current response in case of electro-oxidation of ascorbate itself. Therefore, it can be used to create electrode with electro-catalytically active surface, which helps in lowering the electro-oxidation potential of ascorbate to an appropriate level, nearly to the theoretical limit.

OXIDATION OF ASCORBIC ACID BY ENZYME ASCORBATE OXIDASE

Oxidoreductases are the family to which ascorbate oxidase is belong [17]. These are the group of enzymes those acting on diphenols and related substances as donor with oxygen as acceptor. The systematic name of this enzyme class is L-ascorbate:oxygen oxidoreductase and other names include ascorbic acid oxidase, ascorbase, L-ascorbic acid oxidase, ascorbate oxidase, ascorbic oxidase, ascorbate dehydrogenase. Ascorbate oxidase takes part in ascorbate metabolism. Copper is only the cofactor it employs. It catalyzes the reaction as shown below (Eqn 1):

$$\text{2L-ascorbate} + O_2 \xleftrightarrow{\text{Ascorbate oxidase}} \text{2 dehydroascorbate} + 2H_2O \quad (1)$$

L-ascorbate and O_2 are two substrates whereas dehydroascorbate and H_2O are two substrates of this enzyme. The dehydroascorbate is an electro active species.

BIOSENSORS BASED ON THE IMMOBILIZED ASCORBATE OXIDASE ONTO DIFFERENT INSOLUBLE SUPPORTS

Different types of reusable biosensors based on immobilized ascorbate oxidase are developed for the determination of L-ascorbic acid level in different samples (Table 1). The working of these biosensors based on the reaction catalyzed by ascorbate oxidase as follows (Eqn 2 & 3):

At anode (working electrode):

$$\text{L-Ascorbate} \xrightarrow{\text{Ascorbate oxidase}} \text{Dehydroascorbate} + 2H^+ + 2e^- \quad (2)$$

At cathode (Ag/AgCl electrode):

$$2\,AgCl + 2e^- \xrightarrow{\text{Voltage: 0.6V}} 2\,Ag^0 + 2\,Cl^- \quad (3)$$

As described previously that dehydroascorbate is an electro active species which release a pair of electron when oxidized generate current. The magnitude of the current is proportional to the concentration of ascorbic acid in the sample.

The availability of different types of insoluble supports for enzyme immobilization provides advancement in the fabrication of electrochemical biosensor for ascorbic acid determination. Different types of insoluble support used for the immobilization of ascorbate oxidase include: Collagen membrane [18], CH-Sepharose via carbodiimide [19], Clark-type oxygen electrode [20], oxygen electrode [21], graphite/epoxy electrode [22], gelatin with Teflon membrane [23], cyanogen bromide activated-Sepharose 4B [24], Clark electrode [25], nylon net [26], platinum microelectrode [27], epoxy resin [28], egg shell membrane [29], multiwalled carbon nanotubes/polyaniline modified Au electrode [30], ZnOnanorods [31], Poly(3,4-ethylenedioxythiophene) /Multi-Walled Carbon Nanotubes Composite Films [32], biocompatible poly (3, 4-ethylenedioxythiophene) (PEDOT) matrices [33], amberlite IRA-743 [34].

NANOMATERIALS IN THE FABRICATION OF ASCORBATE OXIDASE BIOSENSORS

In recent years, the use of nanomaterials in electrode modification and electrochemical biosensors development has increased due to its extraordinary properties like high mechanical strength, large surface area, good conductivity and extremely miniaturized size. Many reviews were published on the nanomaterials and their applications. Using nanoscale materials for the fabrication of electrochemical biosensors give rise to major techniques and methods [35]. Recent advances in the synthesis of nanomaterials and its applications were also discussed [36]. Nowadays, different types of nanomaterials are used for the development of new bio-sensing devices. Among all of them, CNTs (Carbon Nanotubes) are more popularly used because of its excellent electrical conductivity, good stability and high mechanical strength. The other advantages of carbon nanotubes is that it is capable to prevent electrode surface fouling when incorporated onto the electrode surface and also enhances the rate of electron transfer during the process [37]. Vairavapandian *et al.* has discussed the various strategies which are used in CNTs preparation and modification [38]. According to Vairavapandian, catalytic behavior of CNTs can be enhanced by the incorporation of metal nanoparticles into the carbon nanotubes matrices [38]. Other than CNTs, there are several other nanoparticles such as ZnO, which are used for the construction and modification of electrodes [31].

Table 1. A brief account of various ascorbate oxidase based biosensors.

No.	Biosensor fabrication	Linear response	Detection limit	Storage life	Reference
1	Ascorbate oxidase immobilized on collagen membrane.	---	---	---	18
2	Immobilized ascorbate oxidase on CH-Sepharose via carbodiimide.	3×10^{-7} and 5×10^{-4} M	---	---	19
3	Clark-type oxygen electrode coupled with slice of mesocarp contains ascorbate oxidase	---	0.02–0.57 mmol l^{-1}	---	20
4	Ascorbate oxidase immobilized on oxygen electrode	concentration range = 6.3×10^{-5} to 5.0×10^{-4} M	6.3×10^{-5}	2 months	21
5	Ascorbate oxidase immobilized in a graphite/epoxy electrode by occlusion in a poly(ethylene-co-vinyl acetate) matrix	8.0×10^{-6} and 4.5×10^{-4} mol l^{-1}	$8.0\text{x}10^{-6}$ M	1 month	22
6	Ascorbate oxidase immobilized with gelatin using glutaraldehyde and fixed on pretreated teflon membrane served as an enzyme electrode	$4.0 \times 10^{-4} - 1 \times 10^{-3}$ M	5.0×10^{-5} and 1.2×10^{-3} M	self life = 2 months	23
8	Ascorbate oxidase immobilized on cyanogen bromide activated-Sepharose 4B	Conc. range = 0 – 2.27×10^{-3}	0.022 μM	----	24
9	Clark electrode	---	----	----	25
10	Ascorbate oxidase immobilized on nylon net and fixed over the polypropylene membrane of the oxygen electrode by an o-ring.	Linear range = $1.2\text{x}10^{-4}$ to $1.0\text{x}10^{-3}$ mol L^{-1}.	---	---	26
11	Ascorbate oxidase based platinum microelectrode prepared by electrochemical etching	---	---	---	27
12	Ascorbate oxidase immobilized epoxy resin	---	---	90 days at 4°C	28
13	Ascorbate oxidase immobilized on egg shell membrane	1×10^{-5} M and 4×10^{-4} M	10 μM	4 months	29
14	Ascorbate oxidase immobilized on multiwalled carbon nanotubes/polyaniline modified Au electrode.	Linear range = 2–206 μM	0.9 μM	2 months at 4 °C	30
15	Ascorbate oxidase immobilized on ZnO nanorods.	Linear dynamic concentration range = 1×10^{-6} to 5×10^{-2} M.	---	---	31
16	Ascorbate oxidase immobilized in Poly(3,4-ethylenedioxythiophene)/Multi-Walled Carbon Nanotubes Composite Films	0.05 to 20 mM	15 μM	1 month	32
17	Ascorbate oxidase (AO) electrochemical biosensor based on the biocompatible poly(3, 4-ethylenedioxythiophene) (PEDOT) matrices	---	---	---	33
18	amberlite IRA-743	1 to 50 μmol L^{-1}	0.14 μmol L^{-1}	----	34

ADVANTAGES OF USING NANOMATERIALS

By knowing the wide range of advantageous properties such as high mechanical strength, large surface area, good conductivity and extremely miniaturized size, nanomaterials are used extensively as support for immobilization of ascorbate oxidase. The various kinds of nanomaterials matrices used in ascorbate biosensors have been discussed in earlier paragraphs.

CONCLUSION

Among all the laboratory based methods, biosensors are more popular and widely used for ascorbic acid determination because it has enhanced specificity and selectivity towards ascorbic acid. Introduction of nanoparticles in electrode fabrication are used as a powerful tool in such applications. Biosensor's miniaturization makes them easy to handle for consumers. With all its properties, biosensors are more reliable, simple, selective and cost effective than many other conventional methods. Commercially available biosensor based diagnostic devices may bring revolutionary changes in the betterment of human health.

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